Modifiers of GRN-associated frontotemporal lobar degeneration

Modifiers of *GRN*-associated frontotemporal lobar degeneration

Eline Wauters[1,2], Sara Van Mossevelde[1,2,3,4], Julie van der Zee[1,2], Marc Cruts[1,2] and Christine Van Broeckhoven[1,2]

[1] Neurodegenerative Brain Diseases, Center for Molecular Neurology, VIB, Antwerp, Belgium

[2] Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

[3] Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerp, Belgium

[4] Department of Neurology, Antwerp University Hospital, Edegem, Belgium

ABSTRACT

Heterozygous loss-of-function (LOF) mutations in the human progranulin gene (*GRN*) cause frontotemporal lobar degeneration (FTLD) by a mechanism of haploinsufficiency. Patients present most frequently with frontotemporal dementia, which is the second most common neurodegenerative dementia at young age. Currently, no disease-modifying therapies are available for these patients. Stimulating GRN protein expression or inhibiting its breakdown is an obvious therapeutic strategy, and is indeed the focus of current preclinical research and clinical trials. Multiple studies have demonstrated the heterogeneity in clinical presentation and wide variability in age of onset in patients carrying a *GRN* LOF mutation. Recently, this heterogeneity became an opportunity to identify disease modifiers, considering that these might constitute suitable targets for developing disease-modifying or disease-delaying therapies.

HETEROGENEITY IN *GRN*-ASSOCIATED FTLD: AN ADVANTAGE OF THE DISADVANTAGE

FTLD refers to a group of neurodegenerative disorders accounting for up to 20% of the neurodegenerative dementia patients younger than 65 years [1] **(BOX 1)**. Patients present with behavioral and personality changes or language disturbances. The prevalence is age-dependent, and overall estimates vary between 2 and 35 per 100,000 [2]. Incidence estimates vary between 1.3 and 4.1, but increase to 16.7 in the age group older than 65 years [2].

Mutations in the human progranulin gene (*GRN*) are a major cause of FTLD, accounting for up to 11.2% of patients. Since the identification of *GRN* as a causal gene for FTLD, studies have demonstrated a marked heterogeneity in clinical presentations associated with mutations in *GRN* [3;4]. For instance, in patients as well as in families segregating the same mutation, multiple clinical presentations have been documented, including the **behavioral variant of frontotemporal dementia** (bvFTD), **primary progressive aphasia** (PPA), **Alzheimer's disease** (AD) and **Parkinson's disease** (PD) (e.g. [5;6]). In addition, highly variable ages of onset ranging from 35 to 89 years have been observed [7;8]. This signifies an important challenge for clinical diagnostics and genetic counseling. The pronounced heterogeneity of disease presentation has led to an incentive to identify *GRN*-related FTLD modifiers, as these might constitute putative targets for developing disease-delaying therapies. This approach is particularly important given that there are currently no cures for FTLD patients. From another angle, other studies are presently investigating the heterogeneity of GRN protein levels in human blood. In these studies, factors have been identified that regulate GRN levels, which may provide candidate targets for drug discovery efforts. Here, we review the heterogeneity of *GRN*-associated FTLD and known disease modifiers. We discuss the approaches that can be used to identify modifiers of clinical heterogeneity and illustrate the progress made to date in therapeutic modification of *GRN*-associated FTLD.

Trends

- Heterozygous LOF mutations in *GRN* leading to GRN protein haploinsufficiency are a major cause of FTLD.
- Ongoing preclinical research and clinical trials are focused on therapies to increase GRN levels or to inhibit its breakdown.
- Patients carrying a causal *GRN* mutation, display phenotypic and age of onset heterogeneity.
- SORT1, PSAP, and RIPK1 have been identified as modifiers of GRN levels.

- Variations in *TMEM106B* have been identified as a risk factor for FTLD-TDP, with a strong effect in *GRN* mutation carriers.
- Research into onset age modifiers of FTLD has intensified. These modifiers are potential targets for disease-modifying therapies, which are currently not available.

BOX 1: Frontotemporal lobar degeneration, a heterogeneous group of disorders

The term FTLD refers to the characteristic atrophy of the frontal and temporal lobes of the brain. FTLD is a proteinopathy and, based on the nature of the inclusion proteins, five pathological subtypes are recognized. The most frequent pathological diagnoses are FTLD-tau and FTLD-TAR DNA binding protein 43 (FTLD-TDP). TDP-43 is localized in the nucleus under normal conditions, whereas in disease, it is aberrantly localized to the cytoplasm [112]. TDP-43 proteinopathy is characterized by pathological TDP-43 modifications such as aggregation, proteolytic cleavage, hyperphosphorylation, and ubiquitination [112]. FTLD-TDP is classified into four pathological subtypes based on the morphology, distribution, density and intracellular localization of inclusions [30], and recently, a fifth type was suggested [113]. Less common are FTLD with inclusions positive for FET-family members FUS (fused in sarcoma), EWS (Ewing's sarcoma) and TAF15 (TATA-binding protein-associated factor 15) (FTLD-FET), FTLD-ubiquitin proteasome system (FTLD-UPS), and FTLD with no inclusions (FTLD-ni) (reviewed in [114]).

Depending on the predominant presenting clinical features, two main subtypes of frontotemporal dementia (FTD) have been defined: behavioral variant FTD with behavior and personality changes, and the non-fluent and **semantic variants of PPA** with language disturbances [115;116]. In addition, a third variant of PPA, the **logopenic variant PPA** has been defined; this subtype is mostly associated with a neuropathological diagnosis of AD [115].

The age of onset of FTD patients is highly variable and ranges from 20 to 91 years [7], with an average of 58 years [117-119]. The median survival from onset varies from 6 to 12 years, independently of age of onset, gender, and education level [120]. Carriers of causal mutations show a high inter- and intra-familial variability of age of onset and clinical phenotype [6;7;23;121]. This clinical heterogeneity suggests that additional genetic or other factors are important contributors to the presentation of disease.

Approximately 25–50% of FTD patients have a family history of dementia, and the disease often segregates in an autosomal dominant manner [122;123]. This points to an important genetic contribution to

disease etiology. Causal mutations have been identified in *GRN*, *C9orf72*, *MAPT*, *TBK1*, *VCP*, and *CHMP2B* (reviewed in [124]).

Genetic and pathological factors overlap with other neurodegenerative brain diseases. *GRN* mutations have been observed in patients clinically diagnosed with AD or Parkinson's disease (PD) [6], and TDP-43 pathology has been documented in FTD, amyotrophic lateral sclerosis (ALS), FTD-ALS, AD, and PD [6;125]. The complex picture of heterogeneous and overlapping genetics, pathological features, and phenotypes challenges the clinical diagnosis and the development and evaluation of therapeutics. Currently, no disease-modifying therapies are available for FTD patients.

GRN-ASSOCIATED FTLD

GRN mutation spectrum

In 2006, heterozygous LOF mutations in *GRN* were identified in families co-segregating autosomal dominant frontotemporal dementia (FTD) linked to chromosome 17q21.31 [3;4]. Mutation screening indicated that LOF mutations in *GRN* accounted for 1–11.2% of all FTD patients and 12.8–25.6% of familial patients [9]. The heterozygous loss of GRN pointed to **haploinsufficiency** as the underlying disease mechanism, which had not been reported before in FTD, or other neurodegenerative brain diseases. In blood and cerebrospinal fluid (CSF), GRN levels are reduced in affected and unaffected carriers [10-12]. The spectrum of *GRN* LOF mutations comprises **frameshift mutations**, **nonsense mutations**, splice site mutations, deletions, and mutations affecting translation and expression (http://www.molgen.vib-ua.be/FTDMutations, [7]). In addition, missense mutations have been found that affect secretion and degradation of the GRN protein, proper protein folding, subcellular localization, proteolytic processing or splicing [13-16]. For certain missense mutations, GRN serum levels are reduced to intermediate levels, lying between those of control individuals and *GRN* LOF carriers, and leading to a partial loss of function [10;17]. For a large number of silent and missense mutations, the contribution to the genetic etiology of FTD is less well understood. In homozygous LOF mutation carriers, complete loss of GRN causes **neuronal ceroid lipofuscinosis (NCL)**, a lysosomal storage disorder [18], or **complicated spastic paraplegia** [19].

Clinical and pathological characteristics

Patients carrying a heterozygous *GRN* LOF mutation mostly present clinically with FTD, and bvFTD is the most common diagnosis [20;21]. Apathy is the prominent behavioral sign, while inappropriate behavior and

agitation are more common in patients with a **repeat expansion mutation in the *C9orf72* gene** [20;22;23]. *GRN* mutation carriers present more frequently with PPA, compared to patients carrying pathogenic mutations in other FTD genes [20;23]. **Extrapyramidal symptoms** are frequent and motor neuron disease (MND) is rare [20;21;24]. The clinical heterogeneity of *GRN* mutation carriers includes AD, PD, PD with dementia, or **corticobasal syndrome (CBS)**. The age of onset is highly variable and an **age-dependent penetrance** has been reported, with mutation carriers dying at an advanced age without apparent clinical manifestation [6;9;25]. Characteristic neuroimaging features consist of asymmetric brain atrophy and early parietal involvement [20;21;26-28]. Macroscopic examination of postmortem brains of *GRN* mutation carriers, has shown neuronal loss in the frontal and temporal cortical areas and degeneration of the parietal lobe [20;29]. Moreover, *GRN* mutation carriers typically exhibit FTLD-TAR DNA binding protein 43 (FTLD-TDP) type A pathology [29-31] **(BOX 1)**. Type A pathology is defined by numerous short thick dystrophic neurites (DNs) and compact neuronal cytoplasmic inclusions (NCIs) which are primarily located in neocortical layer 2 [29-31], the second most superficial layer of the **neocortex**. Furthermore, lentiform neuronal intranuclear inclusions (NIIs) can be present. NCIs, NIIs and DNs are more abundant in upper versus deeper cortical layers [29-31]. These characteristics are relevant as FTLD-TDP type A differs from other types of FTLD-TDP in terms of morphology, distribution, density, and intracellular localization of such inclusions **(BOX 1)**. Type A has been observed in patients with bvFTD or PPA, while for example FTD with MND has been associated with FTLD-TDP type B [32]. The characteristics of *GRN*-associated FTLD are illustrated in **Figure 1**.

Neuroimaging studies and neuropsychological testing of presymptomatic carriers of a mutation in either *GRN,* microtubule-associated protein tau (*MAPT)* or *C9orf72* **(BOX 1)**, have indicated that imaging and cognitive changes are already present in the brain before the expected onset of symptoms [33-35]. These preclinical changes suggest that it would be advisable to intervene with a putative disease-modifying therapy at an early stage of the disease process, and before the onset of clinical symptoms. Thus, accurate and early diagnosis, or even predictive measures, are essential in attempting to curb disease progression.

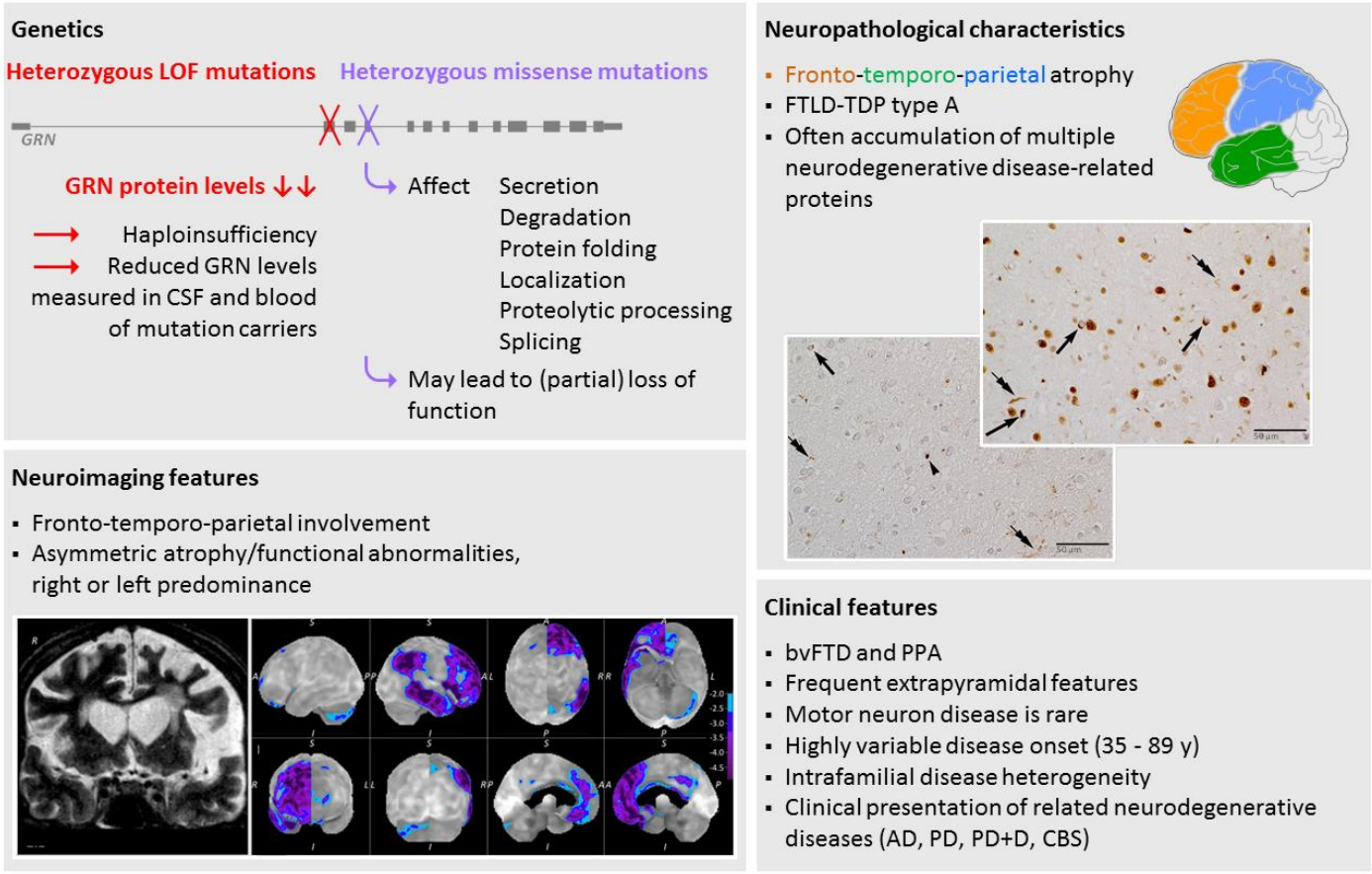


Figure 1: Characteristics of *GRN*-associated FTLD. The figure illustrates the genetics (top left), neuropathological characteristics (top right), neuroimaging features (bottom left), and clinical features (right bottom) of *GRN*-associated FTLD. Structural and functional neuroimaging of *GRN* mutation carriers are shown. Left: coronal MRI T2-weighted image illustrates an asymmetric predominant left atrophy. Right: asymmetric hypometabolism is shown in the right frontal, temporal and parietal lobe (z-map rendering FDG-PET). Neuropathological characteristics: representative paraffin-embedded postmortem FTLD-TDP type A patient brain sections of *GRN* mutation carriers, histologically stained with anti-hyperphosphorylated TDP-43 antibody. Micrographs are: top, Area 6 tissue; bottom, Area 4. Arrows: neuronal cytoplasmic inclusions; double arrows: dystrophic neurites; arrow head: neuronal intranuclear inclusions. Scale bar = 50 µm. AD: Alzheimer's disease, bvFTD: behavioral variant FTD, CBS: corticobasal syndrome, FDG-PET: fluorodeoxyglucose positron emission tomography, FTD: frontotemporal dementia, FTLD: frontotemporal lobar degeneration, FTLD-TDP: FTLD-TAR DNA binding protein 43, GRN: progranulin, MRI: magnetic resonance imaging, PD: Parkinson's disease, PD+D: Parkinson's disease with dementia, PPA: primary progressive aphasia.

GENETIC MODIFIERS

TMEM106B

A **genome-wide association study** (GWAS) identified variations in the transmembrane protein 106B (*TMEM106B)* as a strong risk factor for FTLD-TDP pathology [36]. The minor allele of the single nucleotide polymorphism (SNP) rs1990622 exhibited a lower frequency in FTLD-TDP patients compared to controls, suggesting that individuals expressing the minor allele were less likely to develop disease (odds ratio 0.61) [36]. Stratification based on *GRN* mutation status, showed the highest significance of association in patients with a *GRN* mutation, indicating that *TMEM106B* variants likely exhibit the strongest effect in *GRN* mutation carriers [36;37].

TMEM106B is a single-pass integral membrane glycoprotein, localized in late endosomes or lysosomes. TMEM106B was co-localized with lysosome-associated membrane protein 1 (LAMP1), a late endosomal/lysosomal marker, in HEK293 and Neuro2A cells, and rat and mice cortical and hippocampal neurons (e.g. [38-40]). Overexpression and repression studies in various cell models indicated that TMEM106B is implicated in lysosomal functioning, with overexpression causing an enlargement and poor acidification of endo-lysosomes. Suppression resulted in less complex **dendritic arborization**; this suggests a role for TMEM106B in the regulation of neuronal morphology [39-43]. Conversely, a recent Tmem106b knockout mouse model, indicated that Tmem106b deficiency also causes impairment in lysosomal acidification [44]. These similar results obtained in overexpression and knockout studies suggest that a proper regulation of TMEM106B expression is necessary for proper lysosomal acidification [44]. TMEM106B overexpression also induced nuclear translocation of transcription factor EB (TFEB) in HEK293 cells and mouse cortical neurons [43]. TFEB is a master regulator of lysosomal function. Under conditions of stress, such as lysosomal dysfunction, the protein moves into the nucleus and enhances expression of genes of the CLEAR (coordinated lysosomal expression and regulation) network [45], such as *GRN* and *PSAP*, encoding prosaposin [45-47] **(BOX 2)**. Thus, TMEM106B participates in the sensitivity of lysosomes to stress [43].

Moreover, because the effects of genetic variations in *TMEM106B* are the most pronounced in *GRN* mutation carriers, further studies have investigated the link between TMEM106B and GRN. In *GRN* mutation carriers, genetic variations in *TMEM106B* are associated with plasma GRN levels, or age of onset [37;48], although not in all studies [49;50]. In reports identifying a significant association, later disease age of onset and higher GRN plasma levels are noted in carriers of the *TMEM106B* rs1990622 protective

minor allele [37;48]. Studies are still inconclusive regarding the possible correlation between *TMEM106B* and *GRN* mRNA levels [37;48;51]. For instance, a negative correlation was found in peripheral blood of FTD patients and control individuals [37] and in postmortem brain material of individuals with TDP-43 pathology but without FTLD [51]. Conversely, a study of postmortem brain samples of healthy individuals did not find evidence of a negative correlation [48]. Other studies have reported a link between TMEM106B and GRN by demonstrating co-localization of these proteins in late endo-lysosomes, as evidenced in HeLa cells overexpressing TMEM106B, as well as in non-transfected mouse primary cortical neurons [40;52]. Of note, a study in asymptomatic *GRN* mutation carriers, suggested that *TMEM106B* variants might exert their effects by modulating brain connectivity [53]. Additional studies will be necessary to understand the biological effects of TMEM106B on GRN and granulins, as well as their relevance to FTLD-TDP pathogenesis.

GRN

Genetic variation in *GRN* has been considered as a potential disease modifier. Homozygous carriers of the minor T-allele of rs5848, a common SNP located in the 3' untranslated region (UTR) of *GRN*, present an increased risk of developing FTD [54]. This was shown in a single SNP patient-control association study of rs5848 in groups of clinical FTD patients and pathologically confirmed FTLD-TDP patients without a *GRN* mutation [54]. rs5848 is located in the 3' UTR of *GRN*, within a binding site of the miRNA miR-659. This miRNA binds more efficiently to the *GRN* 3'UTR when the T-allele is present, and consequently inhibits GRN expression [54]. Carriers of the T-allele exhibit reduced GRN levels in CSF and plasma [50;54-56]. However, genetic studies in independent European cohorts of clinical FTD patients and control individuals, did not find an increased frequency of the rs5848 T-allele in FTD patients, which refuted an association of the rs5848 T-allele with increased risk for FTD [57;58]. Further, analysis of genetic variations in the *GRN* wild-type allele of heterozygous carriers of *GRN* p.Arg493*, indicated that carriers of the A-allele of SNP rs9897526 presented with a delayed age of onset [25].

Modification of *GRN* expression has also been observed for miR-107 and miR-29b [59;60]. Specifically, co-immunoprecipitation experiments aiming to identify transcriptome-wide miR-107 targets in H4 cancer cells, have revealed that *GRN* is an important target [59]. For miR-29b, a miRNA target prediction program predicted a binding site in the 3'UTR of *GRN* [60]. These interactions were validated experimentally, identifying changes in intracellular and secreted GRN protein levels upon overexpression or silencing of these miRNAs in cells [59;60]. From another angle, studies of *GRN* promoter methylation provided evidence for hypermethylation in blood cells of FTD patients without known mutations, and in postmortem brain

material of FTLD-TDP patients relative to control individuals [61;62]. The degree of methylation was inversely correlated with *GRN* mRNA expression levels in **lymphoblasts** and in postmortem brain tissue of FTD patients and control individuals [61]. In brain material of FTLD-TDP patients, DNA methyltransferase 3a (*DNMT3a*) mRNA expression was upregulated in comparison to control individuals [61]. Overexpression of DNMT3a in lymphoblasts, led to a decrease of endogenous *GRN* mRNA levels [61]. Treatment of lymphoblasts with a DNMT inhibitor resulted in reduced *GRN* promoter methylation, higher *GRN* mRNA levels and increased levels of secreted GRN. Also, treatment of a murine microglia cell line with a DNMT inhibitor increased *GRN* mRNA expression levels [61]. In addition, different *GRN* transcripts with short or long 5' UTRs exist. As demonstrated in cells expressing *GRN* with and without 5'UTRs, the 5'UTR can control the expression of GRN at transcriptional and translational levels [63].

Modifiers of GRN levels

The high variability of the GRN concentrations reported in blood and CSF, even in healthy individuals, suggests the involvement of other factors to regulate GRN levels. Understanding these mechanisms might lead to novel therapeutic possibilities aiming to upregulate GRN levels.

A GWAS of plasma GRN levels in healthy individuals identified a genetic association between plasma levels and rs646776 and rs611917. Both variations are located near the *SORT1* gene, within and downstream of the *CELSR2* gene [64]. Another variation in intron 1 of *CELSR2*, rs649281, was suggestively associated with *SORT1* mRNA levels in postmortem brain material of control individuals [64]. Moreover, prosaposin (PSAP) was identified as a regulator of sortilin 1 (SORT1)-independent trafficking of GRN to lysosomes **(BOX 2)** [65]. In a family-based GWAS of individuals without dementia, variations in the *PSAP* locus were identified as regulators of GRN plasma levels [66]. As discussed, differences in GRN levels in blood and CSF have also been observed between individuals carrying different genotypes for *TMEM106B* rs1990622 or *GRN* rs5848, though not in all studies [37;48;50;55;56;67]. Consequently, further studies on larger cohorts using standardized protocols for CSF, serum and/or plasma collection, and GRN concentration measurements, may help clarify this matter.

While discussing modifiers of GRN levels in patients with dementia or healthy individuals, we have to consider that only part of the variability in GRN concentrations in CSF can be explained by concentrations in plasma. Specifically, the percentages of GRN levels in CSF explained by plasma levels, have ranged from 6.2 to 29% in different studies [50;56;67]. Meanwhile, a lack of correlation between CSF and serum GRN levels has also been reported [68].

Several studies have investigated whether heterogeneity in age of onset in FTLD patients carrying a *GRN* mutation could correlate with GRN levels in blood or CSF; however, no significant correlation was observed [10;12;50]. The only exception, to our knowledge, is a study that reported an earlier age of onset in FTLD patients that presented the lowest GRN plasma levels [17]. Overall, these observations suggest that there are other modifiers impacting age of onset.

Other molecules have been proposed as potential modifiers of GRN levels. For example, Ca^{2+}-binding chaperone molecules residing in the endoplasmic reticulum (ER), including calreticulin and immunoglobulin heavy chain-binding protein (BiP), as well as members of the protein disulfide isomerase family, including ERp57 and ERp5, interact with mouse Grn, as demonstrated by chemical crosslinking, immunoprecipitation, and mass spectrometry studies in stably transfected HEK293 cells expressing mouse Grn [69]. In these cells, **siRNA**-mediated knockdown of ERp57 and ERp5 decreased the extracellular levels of Grn [69]. Furthermore, an siRNA screening assay against the kinome (subset of genes encoding protein kinases), revealed that silencing of *Ripk1* -an important mediator of cell death- increased intra- and extracellular murine Grn protein levels in Neuro2A cells, but also in microglial-like BV-2 cells, mouse wild-type primary neurons, and neurons from an FTD mouse model [70]. These findings suggest that modulating the ER chaperone network or targeting the receptor-interacting serine/threonine protein kinase 1 (RIPK1) could be exploited to increase extracellular GRN levels, and might represent a potential strategy for treating patients with *GRN*-associated FTLD (presenting GRN haploinsufficiency) [69;70].

Heterogeneity as an opportunity to identify novel modifiers of age of onset and clinical phenotype

It is clear that disease heterogeneity in FTLD complicates clinical diagnosis and therapeutic development. Conversely, the high degree of heterogeneity can be exploited as an opportunity to search for modifiers of age of onset or clinical phenotype, and these modifiers may represent targets for disease-modifying therapies.

A possible approach to identifying genetic modifiers may be to perform GWAS in FTLD cohorts to associate genetic variants with age of onset variability, as an example [71,171]. A potential limitation or challenge to this genetic approach is collecting large cohorts of relatively homogeneous patients to obtain sufficient statistical power. An example is the GWAS in Italian FTD patients, which identified several genetic variations that showed suggestive evidence of association with age of onset [71]. By combining multiple variations, a risk score was generated, explaining up to 14.5% of age of onset

variability [71]. Furthermore, reanalysis of GWAS data [72] using a gene-based test combining multiple variants located in the same gene instead of one single variant, identified genes that were associated with clinical subtypes of FTD, such as *TOMM40* with bvFTD and *SERPINA1* with **progressive non-fluent aphasia** [73].

Another choice is to use a family-based approach, allowing the use of valuable family information to identify genomic loci linked to a defined phenotype or quantitative trait such as age of onset. Here, the challenge is the identification of extended families that are sufficiently informative to reach statistical significance. One example is the extended Belgian founder family co-segregating a causal *GRN* mutation with FTLD, and displaying ages of onset ranging from 45 to 80 years [3;6;10;49;74]. In this founder family, the analysis included data from 85 patients and 40 yet unaffected mutation carriers, identifying a genomic locus that affected age of onset [74]. Comparable approaches have been applied in families segregating AD, leading to the identification of loci associated with age of onset [75].

Additionally, genomics, transcriptomics, proteomics or other '-omics' approaches can be used to compare FTD patients with extreme phenotypes, such as patients with extreme early or late disease onset. Transcriptome-wide differential expression analysis has been carried out on leukocytes from Italian bvFTD and PPA patients carrying the same *GRN* mutation, identifying the gene *RAP1GAP*, which could represent a potential modifier of the clinical phenotype [76]. Another study investigated the shift from asymptomatic to symptomatic FTD disease stage by microarray gene expression analysis on leukocytes of Italian individuals carrying the same *GRN* mutation [77]. Here, a significant increase in *LY6G6F* and *TMEM40* mRNA expression was identified in affected mutation carriers relative to asymptomatic mutation carriers and control individuals [77].

Apart from the hypothesis-free approaches discussed above, targeted studies can be used to investigate the effect of candidate modifiers. One example is the investigation of plasma levels of secretory leukocyte protease inhibitor (SLPI), a serine protease inhibitor which can bind to GRN or elastase and thereby inhibit proteolytic cleavage of GRN into pro-inflammatory granulins [78] **(BOX 2)**. The data revealed increased SLPI plasma levels in affected *GRN* mutation carriers relative to unaffected *GRN* mutation carriers and control individuals, with a later onset of disease in *GRN* mutation carriers harboring the highest levels of SLPI [79]. Others have reported the p.M129V polymorphism in the *PRNP* gene as a modifier of age of onset in *GRN* mutation carriers [80]. FTD patients with at least one V-residue, developed disease on average 8.5 years later in comparison to homozygous carriers of the M-residue.

This finding suggests that this *PRNP* polymorphism might be a possible modifier of the age of onset of *GRN* mutation carriers; however, the data require replication since the patient group size used in this study was small and the statistical finding, potentially spurious [80].

Modifier screens in model organisms or cellular model systems such as **induced pluripotent stem cells (iPSCs)**, are often used to identify modifiers or analyze modifying effects that may be relevant to disease. When a model displays a measurable phenotype, modifier screens can be performed to identify genes modifying the phenotype. For *GRN*, different cellular and animal models, including *Caenorhabditis elegans*, *Danio rerio* and *Mus musculus* models (reviewed in [81]) and patient-derived iPSCs [82;83] have been established, although results of genome-wide modifier screens have not yet been published. Valuable insights may come from the numerous modifier screens that have been performed in TDP-43 model systems [84;85]. Of note, existing *GRN* animal models do not capture all features of FTD, and this should be taken into consideration. Specifically, heterozygous loss of Grn ($Grn^{+/-}$) in mice, causes little to no changes in behavior or neuropathology, while homozygous $Grn^{-/-}$ mice display neuropathological abnormalities such as ubiquitin pathology, increased TDP-43 phosphorylation, **microgliosis**, **astrogliosis**, **lipofuscin deposits**, and subtle behavioral deficits (reviewed in [81]). The reasons for the discrepancy with human disease are unclear, although it has been suggested that the shorter life span of mice prevents accurately modeling the age-dependent neuropathology that is observed in humans [86]. Furthermore, additional modifying factors may be involved in the development of human FTLD-TDP, as suggested by the variability observed in patient age of onset [86]. Generally, the current data indicate that modifier screens are an alternative approach to identifying disease modifiers, though not all *GRN* models show features of human disease.

BOX 2: GRN and its cellular functions

The 12 coding exons of the human *GRN* gene encode the growth factor GRN (also known as granulin precursor, epithelin precursor, proepithelin, PC cell-derived growth factor, GP88, and acrogranin). The protein consists of a signal sequence and seven and a half tandem repeats of a 10 to 12 cysteine-containing motif, the granulin domains. Cleavage of the signal peptide results in mature GRN, which is glycosylated and secreted. Secreted GRN in its native form exists predominantly as a homodimer [66;126]. GRN can be secreted as a soluble protein, but also incorporated into exosomes; a subclass of small secreted vesicles [127]. Cleaving of GRN by proteases elastase, proteinase 3 or certain metalloproteinases, results in the generation of granulin peptides or granulins (granulins A, B, C, D, E, F, G and paragranulin) [78;128-132]. Secretory leukocyte protease inhibitor (SLPI) can bind GRN or elastase, and thereby inhibit

cleavage [78]. Mice that lack the *Slpi* gene show impaired wound healing and increased inflammation, confirming the protective role of SLPI [133]. Recently, cathepsin L, a lysosomal cysteine protease, was shown to cleave intracellular GRN into granulins in HEK293 cells [134;135]. The biological functions of GRN and granulins are not fully understood, but they are believed to be implicated in multiple processes including inflammation, wound repair, and tumorigenesis (reviewed in [81]). Full-length GRN and granulins may have opposing roles in inflammation, with GRN having anti-inflammatory properties and granulins being pro-inflammatory [78]. In the central nervous system, GRN is a neurotrophic factor that enhances neuronal survival and axonal outgrowth and branching [136;137]. An important function of GRN in lysosome biology has been suggested by several studies. For example, *GRN* expression is regulated by TFEB, which also regulates other lysosomal genes [45]. Furthermore, patients with a homozygous LOF mutation in *GRN*, leading to GRN deficiency, develop a lysosomal storage disorder [18]. Grn-deficient mice also display defects in lysosomal turnover and increased lipofuscinosis in the liver and brain (e.g. [138]). Of note, a study showed that GRN deficiency caused an upregulation of lysosomal genes and genes involved in innate immunity in microglia, increasing complement production and synaptic pruning [139].

Sortilin 1 (SORT1), a membrane receptor encoded by the gene *SORT1*, was identified as a high-affinity neuronal receptor of GRN [100;140]. SORT1 mediates GRN endocytosis and guides GRN to the endolysosomal pathway, resulting in reduced extracellular GRN levels [100]. In $Sort1^{-/-}$ mice, Grn levels are increased in the brain and serum [100]. Recently, a SORT1-independent and complementary mechanism for GRN lysosomal trafficking was identified via its interaction with PSAP [65]. Recombinant GRN that is unable to bind SORT1 is not endocytosed when added to fibroblasts or cortical neurons from $Grn^{-/-}$ mice, while adding PSAP leads to lysosomal accumulation of both molecules, indicating that PSAP facilitates lysosomal delivery of GRN from the extracellular space [65]. Further, PSAP also facilitates GRN lysosomal delivery in the biosynthetic pathway [65]. Insulin like growth factor 2 receptor (IGF2R) and low-density lipoprotein receptor-related protein 1 (LRP1), are required for PSAP-mediated GRN lysosomal targeting [65]. Conversely, GRN can enhance neuronal uptake of PSAP via SORT1 [141]. Thus, GRN and PSAP may facilitate each other's uptake and lysosomal trafficking [141]. Moreover, $Psap^{-/-}$ mice exhibit increased serum Grn levels relative to wild-type mice [65]. The identification of these two SORT1- and PSAP-mediated GRN lysosomal trafficking pathways, further supports the lysosomal function of GRN. GRN also binds tumor necrosis factor receptors (TNFR1 and TNFR2), blocking the binding of TNF-α [142]. These interactions have been confirmed in various models and conditions (reviewed by [143]). Lastly, EPHA2, a receptor tyrosine kinase, is identified as a novel receptor for GRN in human urinary bladder cancer cells, umbilical vein endothelial cells, and prostate cancer cells [144].

THERAPEUTIC APPROACHES IN *GRN*-ASSOCIATED FTLD

Upregulating GRN levels

Since *GRN* LOF mutations lead to FTLD due to haploinsufficiency, the obvious first therapeutic approach may be to increase GRN levels, with the aim of correcting haploinsufficiency, and reaching GRN concentrations comparable to those of healthy individuals. Upregulation of GRN levels might be achieved by either gene therapy, administration of recombinant GRN protein or boosting endogenous expression **(Figure 2)**. At this point, however, it is unclear if GRN should be upregulated in all cells, or only in neurons or glia, if upregulation of full-length GRN is necessary to modulate disease, or if upregulating certain granulins could be beneficial. Nevertheless, several approaches aiming to upregulate GRN have been initiated.

For instance, zinc finger nuclease-mediated introduction of *GRN* has been undertaken in iPSCs derived from an FTD patient with a *GRN* mutation (FTD-GRN patient). This approach restored *GRN* transcript levels, as well as the ability of these cells to differentiate into cortical neurons *in vitro*, which was previously inefficient [83]. In another study, the adeno-associated virus (AAV)-driven expression of Grn in neurons of $Grn^{+/-}$ mice, reversed social dominance deficits, as demonstrated in the tube test, in which two mice are placed simultaneously inside a tube [87]. The mouse placing two paws outside the tube is considered to be the loser. The $Grn^{+/-}$ mice in this study showed a low social dominance phenotype. However, AAV-Grn injected $Grn^{+/-}$ mice no longer exhibited low social dominance versus AAV-GFP treated wild-type mice [87]. Furthermore, Grn overexpression corrects lysosomal abnormalities, as shown by normalized LAMP1 expression levels in the prefrontal cortex [87]. Others have used viral vector delivery of *GRN* in an MPTP mouse model of PD [88]. MPTP is a parkinsonism-inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. In these mice, *GRN* delivery elevates GRN expression in nigrostriatal neurons, prevents locomotor deficits, protects against nigrostriatal cell loss, preserves striatal dopamine levels, and reduces cellular apoptosis and inflammation [88]. An alternative approach is to deliver recombinant GRN protein. Specifically, intracerebroventricular delivery of human GRN in rats is reported to lead to increased CSF GRN levels [89].

Other studies have focused on identifying compounds that upregulate GRN levels. For instance, inhibitors of vacuolar ATPases such as bafilomycin A1 and clinically used alkalizing compounds such as chloroquine, have been shown to restore Grn/GRN levels in organotypic cortical slice cultures from $Grn^{+/-}$ mice, as well as in lymphoblasts derived from FTD-GRN patients [90]. Elevated Grn levels most likely

occurred via a posttranscriptional mechanism, given that Grn remained elevated during bafilomycin A1 treatment of Neuro2A cells when transcription was blocked by actinomycin D [90]. Moreover, treatment of Neuro2A and HEK293T cells with inhibitors of vacuolar ATPases, also resulted in increased levels of TMEM106B [38;39]. In a pilot phase 2 clinical study, amiodarone, an alkalizing compound commonly used for treating arrhythmias, was orally administered to five FTD-GRN patients, but exerted no significant effect on serum GRN levels [91]. Furthermore, the disease stage after one year was no different from that of untreated patients [91]. However, given the shortcomings of this study, such as the small number of patients tested, the dosing which was limited to what is commonly used in cardiology settings, as well as the lack of GRN CSF measurements, presently, we cannot exclude the therapeutic potential of amiodarone.

Of note, nimodipine, a calcium channel blocker approved by the FDA to improve the neurological outcome of patients with **aneurysmal subarachnoid hemorrhage**, also increases Grn levels [92;93], as demonstrated by increased hippocampal Grn levels in $Grn^{+/-}$ and wild-type mice treated with nimodipine [93]. However, there are indications that this drug might not be a viable option for treating *GRN*-associated FTLD, since in a phase 1 dose-finding clinical study of eight *GRN* mutation carriers receiving oral nimodipine (clinicaltrials.gov identifier NCT01835665), the GRN concentrations in CSF and plasma were not significantly altered from baseline [93].

Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase (HDAC) inhibitor FDA-approved for the treatment of cutaneous T-cell lymphoma, is a small molecule that leads to enhanced *GRN* transcription [94;95]. In one study, SAHA increased *Grn* mRNA and protein levels in Neuro2A cells and was able to normalize *GRN* mRNA levels in FTD-GRN patient-derived lymphoblasts and fibroblasts [95]. Moreover, in iPSC-derived cortical neurons of *GRN* LOF mutation carriers, SAHA treatment increased *GRN* mRNA levels, as well as intracellular and secreted GRN levels, without affecting neuron viability [94]. These neurons were sensitive to cell stress [82], a phenotype that could not be improved by SAHA treatment [94]. A phase 1 clinical trial using SAHA is planned for mild AD and a phase 1/2 study for Niemann-Pick disease type C1 -a lysosomal storage disorder- has been completed, although the results are not yet available (clinicaltrials.gov identifiers NCT03056495 and NCT02124083, respectively). FORUM Pharmaceuticals has developed a series of HDAC inhibitors and demonstrated that one of these, compound FRM-0334, induces an increase in *GRN* mRNA in rat primary neurons and in FTD-GRN patient-derived lymphoblasts [96]. Thus, a phase 2 clinical trial of FRM-0334 in FTD-GRN patients (clinicaltrials.gov identifier NCT02149160) has been initiated; however no outcome is known.

In addition, a targeted study investigated a library of modulators of the autophagy-lysosome pathway, aiming to identify *GRN* regulators. *GRN* expression is upregulated in human fibroblasts and iPSC-derived neurons from *GRN* mutation carriers, and *in vivo* in a $Grn^{+/-}$ mouse model, in response to trehalose treatment [97]. Trehalose is an FDA-approved disaccharide that induces autophagy independent from mechanistic target of rapamycin (mTOR) [98]. GRN concentrations in human haploid cells deficient in TFEB increase to the same levels as in wild-type cells upon trehalose treatment [97]. Furthermore, trehalose treatment of HeLa cells overexpressing GFP-tagged TFEB indicates that the amount of TFEB in the nucleus does not correlate with GRN expression levels [97]. Consequently, upregulation of GRN by trehalose is not mediated by TFEB [97]. Following trehalose treatment of H4 and human neuroblastoma cells, a dose-dependent increase in *GRN* mRNA is observed; in addition, the trehalose-mediated increase of GRN levels is inhibited upon actinomycin D administration to H4 cells [97]. Together, these findings indicate that trehalose increases GRN expression primarily by increasing *GRN* transcription [97]. While trehalose can upregulate GRN levels, autophagy enhancers have also been considered as putative therapeutic targets for neurodegenerative diseases, given that they can promote the clearance of aggregation-prone proteins **(BOX 3)**. For example, trehalose treatment of HEK293 cells overexpressing TDP-43 and TDP-25, a 25-kDa fragment, has been shown to decrease the levels of TDP-43 and truncated TDP [99]. Targeting TDP-43 may constitute a possible therapeutic avenue for *GRN*-associated FTLD **(BOX 4)**.

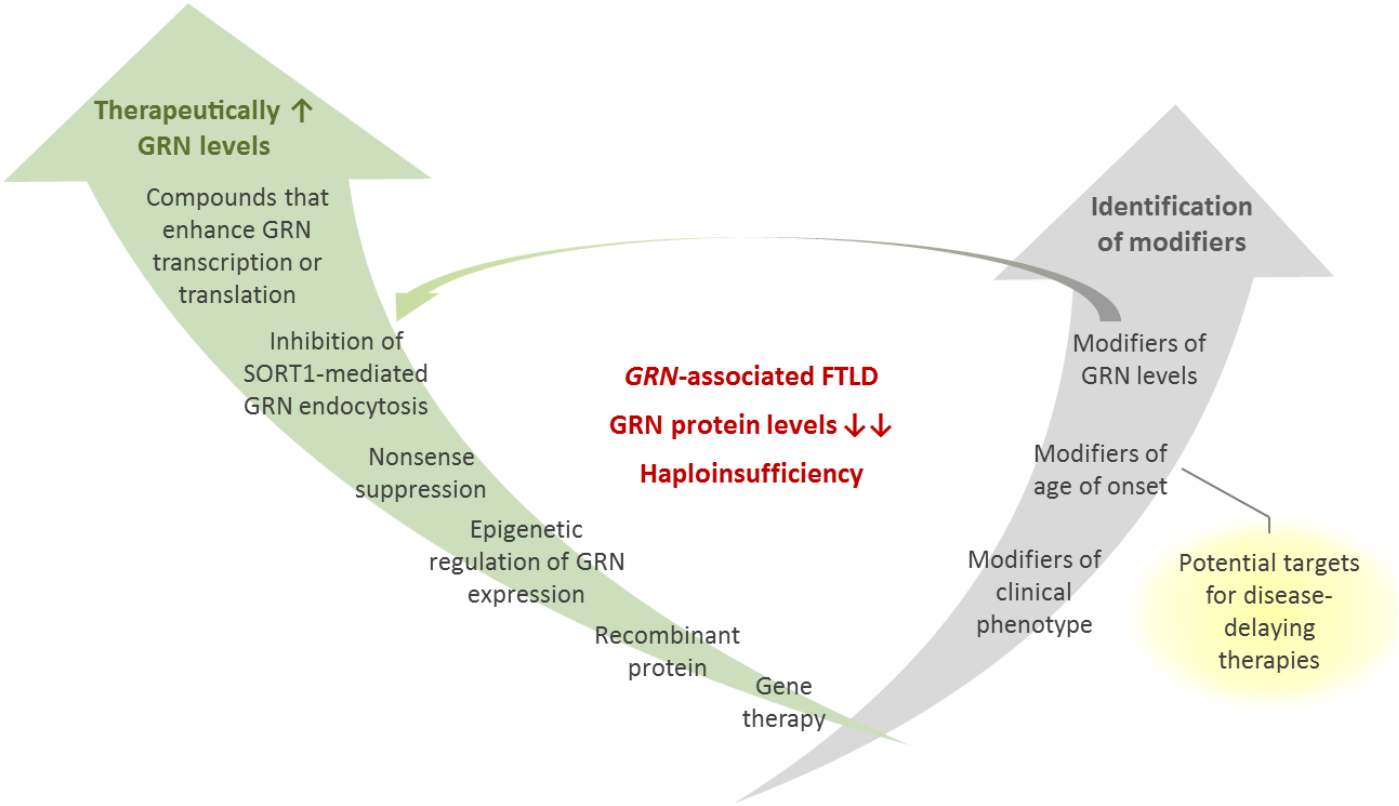
From another angle, *GRN* expression is epigenetically regulated by miRNA binding and promoter methylation. Therefore, we can hypothesize that targeting these epigenetic regulators could include another putative strategy to elevate GRN expression levels; for example by using antisense oligonucleotides to target miRNAs, or by using DNA-demethylating drugs [61].

In addition, approaches to inhibit SORT1-mediated endocytosis of GRN have been explored in an attempt to maintain adequate extracellular GRN levels [100] **(BOX 2)**. Specifically, suppressors of SORT1 expression, SORT1 antagonists or small molecule GRN-binders are three potential strategies under investigation [101]. The small molecule MPEP (1-[2-(2-tert-butyl-5-methylphenoxy)-ethyl]-3-methylpiperidine) is reported to decrease SORT1 levels and increase extracellular GRN levels in mammalian cell lines, and in iPSC-derived neurons and lymphoblasts from FTD-GRN patients [101]. Furthermore, high-affinity SORT1 ligands such as neurotensin prevent access of extracellular GRN to SORT1 binding sites and inhibit GRN endocytosis in SORT1-expressing COS-1 cells [101]. The modulation of GRN-SORT1 interactions via GRN-specific binders might constitute a more promising approach to inhibit

GRN endocytosis, given that SORT1 antagonists could potentially trigger off-target events that cause clinical adverse effects [101]. The small molecule, BVFP (4-[2-(3-bromophenyl)vinyl]-6-(trifluoromethyl)-2(1H)-pyrimidinone), targets a GRN motif that is essential for its interaction with SORT1, and has been shown to reduce the amount of GRN to be captured by SORT1 [101]. Indeed, in human embryonic stem cell lines, BVFP inhibits GRN endocytosis in wild-type cells but not in cells that do not express SORT1, indicating that the effect is SORT1-dependent [101]. However, it is still unclear if inhibition of GRN endocytosis via SORT1 blockade might have deleterious effects on other cell functions, such as those involving lysosomes [101]. Of interest, SORT1 and PSAP have been described as two independent and complementary pathways for GRN lysosomal targeting in both biosynthetic and endocytic pathways **(BOX 2)** [65]. In addition to SORT1, other regulators of GRN levels have been identified (see above), which might also be potential targets for therapeutic intervention in *GRN*-associated FTLD.

Nonsense suppression

A quarter of *GRN* LOF mutations are nonsense mutations (http://www.molgen.vib-ua.be/FTDMutations, [7]), which reduce *GRN* levels by nonsense-mediated mRNA decay of the mutant transcript carrying a premature termination codon (PTC) [3;4]. In this specific case, a therapeutic approach to boost *GRN* expression is based on nonsense suppression, aiming to restore the production of full-length protein. *In*



vitro and *in vivo* studies with compounds such as ataluren (Translarna; PTC Therapeutics), have shown **ribosomal read-through activity** of PTCs in various disease models, including fibroblasts and lymphoblasts from infantile NCL patients (neurological disorder), where production of the full-length protein and increased activity of the enzyme encoded by the mutated gene have been demonstrated [102]. Moreover, in Europe, ataluren has been given a conditional approval for the treatment of Duchenne muscular dystrophy patients (caused by a nonsense mutation), aged five years and older who are able to walk. Accordingly, studies with read-through compounds for patients carrying a *GRN* nonsense mutation are envisioned, but no data are yet available [103].

Figure 2: Modifiers of *GRN*-associated FTLD and therapeutic outlook. The diagram evokes known approaches to upregulate GRN levels as a potential therapeutic strategy to treat GRN-associated FTLD; patients afflicted exhibit reduced GRN protein levels, leading to haploinsufficiency. Clinical heterogeneity as well as heterogeneity in CSF and blood GRN levels may offer opportunities to identify putative modifiers that may represent possible novel

targets for drug development. CSF: cerebrospinal fluid, FTLD: frontotemporal lobar degeneration, GRN: progranulin, SORT1: sortilin 1.

BOX 3: Targeting the protein degradation machinery

Many neurodegenerative brain diseases including FTLD, are characterized by the accumulation of misfolded and ubiquitinated proteins into neuronal aggregates. This suggests that there are defects in protein quality control systems and clearance machinery, or that the demand on degradation mechanisms is too high given the accumulation of aggregated and possibly toxic proteins [145;146]. Consequently, strategies to enhance cellular waste clearance systems, such as the chaperone network, the ubiquitin-proteasome system and/or the autophagy-lysosome pathway, might be beneficial, not only for *GRN*-associated FTLD, but also for other neurodegenerative diseases.

One system to clear misfolded proteins is the proteasome, which degrades ubiquitinated proteins. A small molecule inhibitor of the de-ubiquitinase USP14 promotes TDP-43 clearance in primary mouse embryonic fibroblasts [147].

Autophagy can be induced by mTOR inhibitors such as rapamycin or mTOR-independent upregulators such as trehalose or carbamazepine [145]. In a mouse model with TDP-43 proteinopathy, rapamycin treatment rescued learning/memory impairment, ameliorated motor neuron function, and increased clearance of TDP inclusions [148]. In the same model, carbamazepine rescued motor dysfunction, reduced TDP-43 inclusions, and enhanced neuronal survival [148]. Administration of rapamycin in a *Drosophila* model for TDP-43 reduced the number of neurons with aggregates of the *Drosophila* TDP-43 orthologue, with beneficial effects on locomotor defects and lifespan [149]. TFEB is a master regulator of the autophagy-lysosome pathway and upregulation of TFEB has had beneficial effects in models of related neurodegenerative diseases (e.g. [150-152]). Given these encouraging results, TFEB activation might also be an interesting approach to treating FTLD-TDP.

Chaperone proteins are also possible therapeutic targets. These proteins regulate proper protein folding or deliver misfolded proteins for degradation. Activation or overexpression of the transcription factor heat shock factor 1 (HSF1) increases the expression of chaperones [153]. In HEK293 and SH-SY5Y cellular models of TDP-43 proteinopathy, HSF1 overexpression reduces pathological TDP-43 and enhances cell survival [154;155]. Potentiated heat shock protein (Hsp) 104 variants eliminated cytoplasmic TDP-43 aggregates and promoted nuclear localization in yeast [156]. Whether Hsp104 can be introduced as a putative therapeutic remains unclear [156]. Incubation of HeLa cells with an Hsp90 inhibitor reduces levels

of full-length and cleaved TDP-43 [157]. It also prevents reactive oxygen species-induced TDP-43 polymerization and disassembles TDP-43 aggregates in HEK293T cells overexpressing mouse Tdp-43 [158]. Upregulation of the small heat shock protein HSPB8 enhances autophagy-mediated clearance of TDP-43 and truncated TDP in a mouse motor neuron-like cell line and SH-SY5Y cells transiently overexpressing TDP-43 or TDP-25 [159].

BOX 4: Targeting TDP-43, a therapeutic strategy for GRN-related FTLD?

The identification of TDP-43-rich inclusions in the brain of FTD-GRN patients, and the correlation of the distribution of TDP-43 pathology with areas of neurodegeneration, suggest that inhibiting TDP-43 aggregation and toxicity might be a possible therapeutic intervention for *GRN*-related FTLD [160]. TDP-43, encoded by the *TARDBP* gene, is a conserved nuclear RNA-binding protein that functions in transcription and splicing regulation [161;162]. Mutations in *TARDBP* are a more frequent cause of ALS but are rare in FTD patients, thus in most FTLD-TDP patients the wild-type protein is aggregated and located in the cytosol [163]. The pathogenicity of these aggregates and of protein modifications, such as hyperphosphorylation and cleavage into C-terminal fragments, is poorly understood (reviewed in [164]). A possible disease mechanism might constitute a loss of regular nuclear functions of TDP-43, due to nuclear depletion and cytosolic accumulation [112]. Several studies in animal models argue that a loss of TDP-43 function is indeed key in TDP-related diseases (reviewed by [165]). Further, aggregated TDP-43 might be toxic for the cell, although this is a matter of debate [164]. Of note, in sporadic ALS patients with long disease duration, the number of neurons with inclusions has been reported to be lower relative to that in other ALS patients [166]. However, this observation does not rule out that the aggregates are a mere effect of other toxic processes [164]. Whether the nuclear loss of function or a cytosolic gain of toxic aggregated TDP-43, is the major culprit, is currently undecided (reviewed in [164]). Studies have been performed to identify modifiers of TDP-43 phosphorylation, drugs that modify TDP-43 aggregation and pathways controlling TDP-43 nucleocytoplasmic shuttling (e.g. [84;167-169]). These and other possible avenues for TDP-43-related therapeutic strategies are being investigated, including agents that induce protein degradation systems (e.g. proteasome pathway or autophagy) **(BOX 3)** (reviewed in [170]).

TOWARDS EARLY AND TARGETED INTERVENTION

When disease-modifying therapies do become available and a patient is diagnosed with FTD, the question remains on how far the neuronal loss has progressed, and whether it is possible to realize an

effective therapy. To achieve early detection and intervention, biomarkers are needed to monitor progression in the presymptomatic period, disease onset and staging of the disease process. There are however many considerations: who should be screened and when? How will the results be communicated to the patient and what would the ideal timing be for therapeutic intervention to be effective? Furthermore, when presymptomatic individuals enter a clinical trial, some might be at a stage that is years before onset, while others might develop disease within a year. Therefore, it might be necessary to consistently monitor changes in participants in order to account for this type of heterogeneity. In this context, the identification of age of onset modifiers and the development of genetic scores to predict age of onset might be useful [71;104]. For *GRN*-associated FTLD, neuroimaging has shown the potential to serve as a biomarker of disease progression in the presymptomatic stage [33-35]. Determining which individuals to include is easier in families segregating a known mutation. For AD, large GWAS have advanced our knowledge of risk variations, and genetic risk scores can be calculated to identify individuals at high risk who might benefit from certain therapies [105].

The combination of genetics, CSF, and blood and imaging markers would not only be helpful in the identification of individuals at risk of developing FTD and in diagnostic purposes, but also potentially aid to reduce the heterogeneity of clinical trials. As previously mentioned, ongoing clinical trials are already focusing on a specific subgroup of FTD patients so as to perform targeted trials in a more homogeneous patient population; these include patients carrying mutations within the same gene. However, to enable well-defined homogeneous patient groups, further research into FTD genetics, neuroimaging and CSF or blood biomarkers will be necessary.

For novel disease-modifying therapies to be evaluated and applied in the clinic, we also need biomarkers to assess the efficiency of novel drugs over time. For example, GRN levels in CSF might be used as a potential biomarker to monitor the effects of therapies on FTD-GRN patients [106], in addition to neuropsychological testing and neuroimaging. However, caution is warranted since GRN oligomers might not be detected using currently available ELISAs [66]. Recent studies have measured higher levels of neurofilament light chain (NfL) in the serum and CSF of FTD-GRN patients compared to presymptomatic mutation carriers and healthy individuals [107;108]. In these patients, NfL levels correlate with disease severity, brain atrophy and survival [107;108]. Thus, NfL levels might constitute a promising biomarker for disease onset, severity and survival, though further research is necessary to further evaluate the use of NfL levels in clinical trials [107;108].

CONCLUDING REMARKS

FTLD is a devastating disease for which only symptomatic treatments are currently available. With an increased understanding of the genetics and pathomechanisms of FTLD, research focus is shifting towards the development of targeted therapies as well as towards the identification of modifier genes **(BOX 5)**. For *GRN*-associated FTLD, therapeutic strategies are mainly focused on identifying approaches to upregulate *GRN* expression and increase extracellular GRN levels. Specific drugs are currently being tested in clinical trials for this purpose. However, it remains unclear which GRN species are present in the human brain under different conditions and what their role is (see Outstanding Questions).

Examining the variability in GRN levels has resulted in the identification of SORT1 as a regulating factor, and possible therapeutic avenues involving SORT1 are being investigated. New studies have also identified *RIPK1* and *PSAP* as modifiers of GRN levels, offering other novel potential therapeutic targets in FTLD.

In this review, we highlighted the potential of exploiting the clinical heterogeneity associated with *GRN*-associated FTLD to identify disease modifiers, which may represent novel targets for disease-modifying therapies. Studies have begun to identify age of onset modifiers of *GRN*-related FTLD. As putative targets, these modifiers may hold promise for the development of disease-delaying therapies, given that they can alter biological processes in a way that delays or accelerates FTLD pathogenesis. Evidently, multiple factors may be involved in shaping disease presentation, including genetic, epigenetic, and environmental modifiers. In this regard, a study in presymptomatic *GRN*, *MAPT*, and *C9orf72* mutation carriers and non-carriers, has indicated that education level has an effect on grey matter volume; the greater the number of years of education, the greater the volume [109]. This relationship is also modulated by *TMEM106B* rs1990622 genotype in mutation carriers [109]. Furthermore, education and occupation might modulate functional connectivity in the brain [110].

Although the effect of one single modifier may thus be limited, it is conceivable that pharmaceuticals that target an involved pathway have a stronger impact. Additionally, by targeting specific pathways, these therapies might be of broader use than only in patients who carry the modifying allele [111]. Therefore, we embrace the clinical heterogeneity of *GRN*-associated FTLD by viewing it as an opportunity to identify disease modifiers. We hypothesize that this may offer potential treatment strategies where currently no disease-modifying therapies are available.

BOX 5: Clinicians' Corner

- The phenotypic heterogeneity observed in patients with a *GRN* mutation suggests that neurodegenerative brain diseases are a continuum of disorders, overlapping in genetics, pathological features, and clinical phenotypes.
- Appreciating the disease heterogeneity associated with *GRN* mutations is of major importance for genetic counseling. Within families, clinical heterogeneity could mask inheritance of an underlying genetic defect. The diagnosis and prognosis of a preclinical *GRN* mutation carrier cannot be predicted based on the phenotypic characteristics of relatives.
- No disease-modifying therapies are available for patients with *GRN*-related FTD. Current preclinical research and clinical trials are mainly focusing on upregulating GRN levels as a potential therapy.
- Identification of age of onset modifiers is expected to indicate novel targets for disease-modifying therapies.

Outstanding Questions

- How are GRN, granulins, and other structures involving GRN functioning in the brain? Is it necessary for therapeutic strategies to upregulate full-length GRN or is it sufficient to enhance the expression of certain granulins in order to modify disease outcomes?
- Is it necessary to correct GRN expression in neurons, in microglia, or in all cells?
- In certain pedigrees, the observed clinical heterogeneity in patients may be due to the presence of other pathogenic mutations in genes associated with neurodegenerative brain disease. The use of high-throughput sequencing technologies, has enhanced the probability of identifying carriers of a second pathogenic mutation. Consequently, the clinical presentation in these carriers could be confounded due to presence of different underlying pathologies.
- Approaches to identifying modifiers of *GRN*-associated FTLD may lead to loci or genes involved in modification of disease presentation. A major challenge will be to identify the functional genes and variants and to translate these findings into putative therapeutic strategies.

GLOSSARY

Age-dependent penetrance: age-dependent probability that a genotype results in a phenotype; that is, that a carrier of a pathogenic mutation presents with disease.

Alzheimer's disease (AD): most common form of neurodegenerative dementia, characterized by progressive deterioration of brain functions, including memory, thinking, and behavior.

Aneurysmal subarachnoid hemorrhage: bleeding into the compartment surrounding the brain, between the arachnoid membrane and the pia membrane (subarachnoid space) due to rupture of a brain aneurysm.

Astrogliosis: astrocyte responses to insults, characterized by alterations in gene expression and cell proliferation.

Behavioral variant of frontotemporal dementia (bvFTD): The major subtype of frontotemporal dementia, characterized by progressive changes and deterioration in behavior, personality, and cognition.

Complicated spastic paraplegia: characterized by lower extremity spasticity and weakness. The impairment is accompanied by other systemic or neurologic abnormalities such as ataxia, epileptic seizures, mental retardation, cognitive impairment or dementia, thus classified as complicated.

Corticobasal syndrome (CBS): progressive neurological disorder that may involve the motor system, cognition, or both.

Dendritic arborization: process of forming new dendritic trees and branches by neurons.

Extrapyramidal symptoms: involuntary movements, alterations in muscle tone, and postural disturbances.

Frameshift mutation: causes a translation shift to another reading frame.

Genome-wide association study (GWAS): population-based study to identify genetic variations associated with a trait or disease by evaluating genome-wide genetic variation.

Haploinsufficiency: condition where a single functional copy of a gene does not produce enough gene product to maintain a healthy state in a diploid organism.

Induced pluripotent stem cells (iPSCs): type of pluripotent cells that can be obtained by reprogramming differentiated cells. iPSCs can be differentiated into mature cell types such as neurons.

Lipofuscin deposits: fluorescent material that accumulates with age in the lysosomal compartment of postmitotic cells. Also known as the aging pigment.

Logopenic variant PPA: subtype of primary progressive aphasia characterized by impaired single-word retrieval in spontaneous speech and naming, and impaired repetition of sentences and phrases. Additional characteristics include phonological errors in speech, spared single-word comprehension, object knowledge and motor speech, and the absence of frank agrammatism.

Lymphoblasts: immortalized lymphocyte cells through Epstein-Barr virus transformation.

Microgliosis: microglia responses to insults, characterized by changes in morphology, gene expression, and increased local densities of microglia.

miRNA: RNA molecule of ~22 nucleotides binding complementary motifs in mRNA sequences, and leading to target cleavage or translational repression.

Neocortex: represents the majority of the cerebral cortex of the human brain. It consists of layers I–VI, from superficial to deep, principally segregated by cell type and neuronal connections.

Neuronal ceroid lipofuscinosis (NCL): group of neurodegenerative, lysosomal storage disorders characterized by progressive intellectual and motor deterioration, visual failure, epileptic seizures, and early death.

Nonsense mutation: converts a codon that encodes an amino acid into a stop codon.

Parkinson's disease (PD): neurodegenerative brain disorder characterized by bradykinesia, muscle rigidity, resting tremor, postural instability, and nonmotor symptoms such as depression, sleep disturbances, and constipation.

Primary progressive aphasia (PPA): disorder characterized by progressive deterioration of speech and/or language function with an insidious onset. Classified into subtypes: non-fluent/agrammatic variant PPA, semantic variant PPA, and logopenic variant PPA.

Progressive non-fluent aphasia: subtype of primary progressive aphasia characterized by effortful speech, agrammatism, and speech apraxia with initial relative preserved (single-word) comprehension and object knowledge.

Repeat expansion mutation in the *C9orf72* gene: causal expansion of a non-coding G_4C_2 hexanucleotide repeat in the *C9orf72* gene. The size of the repeat ranges from two to >4000 repeat units. Most healthy individuals have repeat sizes below 24 repeat units.

Ribosomal read-through activity: nonsense mutations introduce a stop signal, causing the ribosome to prematurely terminate its reading of the mRNA. Compounds with ribosomal read-through activity are thought to interact with the ribosome in such a way that it reads through the stop signals and produces a full-length protein.

Semantic variant of PPA: subtype of primary progressive aphasia characterized by impaired confrontation naming and single-word comprehension, while speech fluency and grammar are relatively spared.

siRNA: double-stranded RNA molecule of 20–25 base pairs. siRNAs are a common tool for silencing the expression of specific genes.

ACKNOWLEDGMENTS

The research in the authors group was in part funded by the Belgian Science Policy Office Interuniversity Attraction Poles program; the Flemish Government-initiated Methusalem excellence program; the Flemish Government-initiated Impulse Program on Networks for Dementia Research; the Alzheimer Research Foundation; the Research Foundation Flanders (FWO), the Agency for Innovation by Science and Technology Flanders (IWT) and the University of Antwerp Research Fund; Belgium. E.W. received a PhD fellowship of the IWT.

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